Original Contributions

Molecular and Immunohistochemical Analysis of HER2/neu Oncogene in Synovial Sarcoma

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Amplification and/or overexpression of HER2/neu have been documented in many types of epithelial tumor and recently has been reported in sarcomas, particularly in osteosarcomas. But the role of HER2/neu alterations in soft tissue tumors remains poorly understood. Thus the present study investigates the expression of HER2/neu in 13 patients with synovial sarcoma (SS). In this study, HER2/neu mRNA levels were measured in frozen tissue samples using a real-time reverse transcription-polymerase chain reaction assay; protein expression was assessed by immunohistochemistry using an anti-HER2/neu polyclonal antibody. Six normal skeletal muscle specimens were used to establish basal levels of HER2/neu mRNA. HER2/neu transcripts were detected in all normal tissues and SSs. Four of 13 sarcomas (31%) demonstrated HER2/neu mRNA levels above the mean value, whereas 3 tumors (23%) displayed HER2/neu protein overexpression. Both membranous and cytoplasmic patterns of immunostaining were observed, and a strong correlation was found between protein expression and mRNA level ($P = 0.01$). Increased HER2/neu mRNA levels were significantly associated with a lower risk of developing recurrences ($P = 0.02$). Moreover, none of the patients with HER2/neu overexpression developed metastasis. Our data demonstrate that HER2/neu is expressed in SSs and that both membrane and cytoplasmic HER2/neu expression correlate with mRNA levels. Our results show that the presence of increased levels of HER2/neu in SSs is associated with a more favorable clinical course. Further studies are needed to assess the role of this oncogene in SSs and to evaluate the application of inhibitory humanized monoclonal antibodies in the treatment regimens for this malignancy. HUM PATHOL 34:639-645.

Key Words: **HER2/neu, synovial sarcoma, real-time RT-PCR, immunohistochemistry.**

***Abbreviations:* *FISH, fluorescence in situ hybridization*, RT-PCR, reverse transcription–polymerase chain reaction, SS, synovial sarcoma.**

Synovial sarcoma (SS) is an aggressive soft tissue tumor that accounts for up to 10% of sarcomas, with a peak incidence in adolescents and young adults. This tumor occurs in 2 major forms, biphasic and monophasic, and it is cytogenetically characterized by the t(X; 18)(p11;q11) translocation, found in >95% of cases. Although traditionally considered to be a high-grade neoplasm, recent investigations have suggested that different factors influence prognosis, including morphological and cytogenetic features, treatment strategies, the ploidy status, and the apoptotic index.[1]

The development of new therapeutic advancements, such as the specific targeting of molecular alterations present in human malignancies, has brought to light the need to identify not only prognostic factors, but also tumor features that are predictive of response to therapy.

One of the most extensively studied molecular targets for therapy is the HER-2/neu proto-oncogene. The HER-2/neu oncogene (also known as c-erbB-2), located on chromosome 17q21, is a member of the tyrosine kinase receptor family and encodes for a 185-kilodalton protein that shows 50% homology with the epidermal growth factor receptor.[2,3] This gene is amplified and/or overexpressed in 20% to 30% of breast carcinomas[4,5] and in various other tumors,[6] and usually is associated with tumor aggressiveness and poor prognosis.[7,8] Several studies have supported the value of HER-2/neu to predict the response to chemotherapy in breast cancer, and the use of recombinant humanized antibodies to HER-2/neu protein (Trastuzumab) in the care of patients with advanced, metastatic breast tumors has been approved.[9]

The role of HER-2/neu activation in soft tissue tumors remains poorly understood, and scarce molecular data backing immunohistochemical studies have been reported. HER-2/neu protein expression was immunohistochemically studied in 204 sarcomas, including 6 SSs, and overexpression was absent in all these malignant mesenchymal neoplasms.[10]

Recently, HER-2/neu alterations have been described in osteosarcoma, with a high incidence of pro-

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TABLE 1. Clinicopathologic Features of 13 Patients With SS

No.	Age(y)/G	Site	Subtype	Surgery	Chemo/Radio	Rec	Mets	HER2	FU(mo)
1	68/F	KI	MF	RR	No	Yes	Yes	L	108
2	71/F	KE	BF	WA	No	No	No	L	12
3	27/M	KI	BF	ME	Yes	Yes	Yes	L	24
4	39/M	T	MF	WE	Yes	No	No	H	48
5	15/M	N	MF,PD	WE	Yes	No	No	H	48
6	41/F	F	MF,PD	WA	Yes	No	No	H	48
7	57/F	KI	MF	WA	No	N/A	N/A	L	N/A
8	48/M	F	BF,PD	ME	Yes	Yes	No	L	36
9	29/F	T	PD	WE	Yes	Yes	Yes	L	36
10	27/M	KE	BF	WE	No	No	No	H	24
11	62/F	A	MF	WE	No	Yes	No	L	24
12	62/F	F	MF	WA	No	No	No	L	2
13	34/F	L	MF	RR	No	No	No	L	2

Abbreviations: Age, age at diagnosis; G, gender; M, male; F, female; Site, anatomic location; KI, knee, intra-articular; KE, knee, extra-articular; T, thigh; N, neck; F, foot; A, arm; L, leg; Subtype, histological subtype; MF, monophasic fibrous; BF, biphasic; MF-PD, monophasic fibrous with poorly differentiated areas; BF-PD, biphasic with poorly differentiated areas; PD, poorly differentiated; Surgery, primary surgical therapy; RR, radical en bloc resection; WA, wide through-bone amputation; ME, marginal en bloc excision; WE, wide en bloc excision; Chemo/Radio, adjuvant postoperative chemotherapy and/or radiotherapy; Rec, recurrence; Mets, presence of metastasis; HER2, HER2 mRNA expression; L, low expression; H, high expression; FU, follow-up status; N/A, not avaible.

tein expression, ranging from 42% to 61%.[11-15] Indeed, despite limited information on Her2/neu in this type of malignancy, based mostly on immunohistochemical findings, 2 clinical trials of Trastuzumab have been initiated for recurrent and metastatic osteosarcoma patients (http://www.cancer.gov/clinical_trials: MSKCC-99097/NCI-T98-0083 and COG-AOST0121).

Therefore, we evaluated the mRNA expression and the gene product of HER-2/neu in 13 SS patients using real-time reverse transcription–polymerase chain reaction (RT-PCR) and immunohistochemistry. To the best of our knowledge, this is the first report documenting increased levels of HER-2/neu mRNA and protein in SS.

MATERIALS AND METHODS

Patient Population

Thirteen patients with primary SSs, obtained from the files of the Department of Pathology, Gaetano Pini Orthopedic Institute, were included in this study. Cases were chosen based on the availability of frozen primary tumor. Patient age ranged from 15 to 71 years (mean, 44.6 years). Anatomic sites included the knee (5 patients, 3 intra-articular), thigh (2 patients), foot (3 patients), arm (1 patient), neck (1 patient), and lower leg (1 patient). The histological subtypes were 3 biphasic (BF), 6 monophasic fibrous (MF), 2 monophasic fibrous with poorly differentiated areas (MF-PD), 1 biphasic with poorly differentiated areas (BF-PD), and 1 poorly differentiated (PD). Clinical staging was IIB for all the patients. Local surgical excision was performed in 9 patients; amputation, in 4 patients. Recurrence was observed in 5 cases with subsequent amputation (3 patients) and local excision (2 patients). Metastasis occurred to lung (2 patients) and inguinal lymph nodes (1 patients). Five patients received adjuvant postoperative chemotherapy, and 1 patient (case 4) was treated with chemotherapy associated with radiotherapy. Follow-up ranged from 2 to 108 months (mean, 34.3 months). Clinicopathologic data are summarized in Table 1.

Pathologic Studies and Frozen Tissue Selection

In all cases, the primary tumor was available for study. Surgically resected tumor tissues were partly snap-frozen in liquid nitrogen and stored at −80°C for RNA extraction, and partly fixed in buffered formalin and embedded in paraffin blocks. Hematoxylin and eosin–stained sections were re-evaluated and graded according to FNCLCC grading system.[16] Frozen tissue blocks were handled as follows: 4-μ-thick frozen sections were cut and stained with hematoxylin and eosin to determine the percentage of tumor cells present in the specimen. We used tissue blocks with tumor cells comprising more than 80% of the specimen. About 10 20-μ-thick sections were collected into Eppendorf tubes. Another 4-μ-thick frozen section was cut after the serial sections and examined by light microscopy to guarantee the percentage of tumor cells collected. Trizol (Life Technologies; Gibco BRL, Gaithersburg, MD) was used for RNA extraction, according to the manufacturer's protocol. RNA was quantified spectrophotometrically.

cDNA Synthesis

Total RNA (200 ng) was reverse-transcribed in a total volume of 50 μL containing 1 × TaqMan buffer, 5.5 mmol $MgCl_2$, 1 mmol deoxynucleotides, 2.5 μmol random hexamers, 20 U RNase inhibitor, and 62.5 U MuLv reverse transcriptase. The samples were incubated at 25°C for 10 minutes, 48°C for 30 minutes and 95°C for 5 minutes.

PCR Amplification

Amplification reactions were performed with the Universal TaqMan 2 × PCR mastermix in a volume of 25 μL containing 300 nmol of each primer, 100 nmol of probe, and 5 μL of cDNA. Both β-actin and HER2/neu amplification were done in duplicate for each sample.

The thermal cycling conditions included 2 minutes at 50°C and 10 minutes at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. All reagents used for

RT-PCR were purchased from Applied Biosystems (Foster City, CA).

Primers and Probes

Primers and probes for β-actin and HER2/neu mRNA were chosen using the computer program Primer Express (Applied Biosystems). Sequences of the forward primer for HER2/neu mRNA (GenBank accession number X03363) were 5'-TCC TGT GTG GAC CTG GAT GAC-3' and the reverse primer 5'-CCA AAG ACC ACC CCC AAG A-3'; the sequence of the TaqMan probe was 5'(FAM)-AGC AGA ATG CCA ACC ACC GCA GA-(TAMRA)-3'. Sequences of the forward primer for β-actin mRNA (GenBank accession number X00351) were 5-TCC TTC CTG GGC ATG GAG-3' and the reverse primer 5'-AGG AGG AGC AAT GAT CTT GAT CTT-3'; the sequence of the TaqMan probe was 5'(FAM)-CCT GTG GCA TCC ACG AAA CTA CCT TC-(TAMRA)3'. Probes were purchased from Applied Biosystems.

Real-Time RT-PCR

To measure HER2/neu expression in these tumors we used a real-time quantitative RT-PCR based on TaqMan methodology, as previously described,[17] with minor modifications. Briefly, this technique allows, by means of fluorescence emission, to find the cycling point when PCR product is detectable (Ct value or threshold cycle). As previously reported, the Ct value correlates to the starting quantity of the target mRNA.[18] To normalize the amount of total RNA present in each reaction, we amplified the housekeeping gene β-actin, which is assumed to be constant in both normal samples and tumor tissues.

Our results are expressed as relative levels of HER2/neu mRNA, referred to a sample, called a "calibrator," chosen to represent 1X expression of this gene. The calibrator was a breast cancer cellular line (MCF-7)[19] that was analyzed on every assay plate with the unknown samples. All of the analyzed tumors expressed n-fold HER2/neu mRNA relative to the calibrator.

The amount of target, normalized to an endogenous reference (β-actin) and relative to the calibrator, was defined by the ΔΔCt method as described by Livak K (Sequence Detector User Bulletin 2; Applied Biosystems). Specifically, the formula is applied as follows:

$$\text{target amount} = 2^{-\Delta\Delta Ct}$$

where ΔΔ Ct = {[Ct (HER2/neu sample) − Ct (β-actin sample)] − [Ct (HER2/neu calibrator) − Ct (β-actin calibrator)]}.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were deparaffinized, rehydrated, and exposed to the primary antibody using the EnVision+ system (Dako, Carpinteria, CA). Primary anti–HER-2/neu antibody (rabbit polyclonal antibody, catalog number A0485; Dako) was applied in a dilution of 1:2000 for 60 minutes at room temperature. Before exposure to the primary antibody, sections were microwave-pretreated in EDTA, pH 8.0, to retrieve antigenicity, and incubated with endogenous peroxidase-blocking solution for 10 minutes at room temperature. Positive control, constituted by a breast carcinoma showing more than 80% positive staining for HER2/neu, as well as negative control, in which the primary antibody was omitted, were stained in parallel.

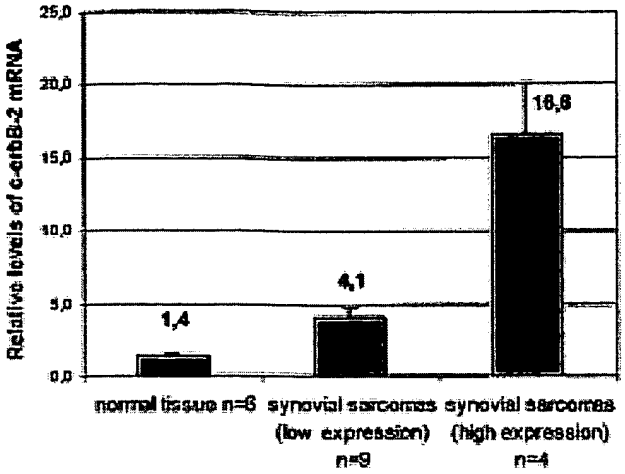

FIGURE 1. Distribution of HER2/neu mRNA levels in normal muscle tissues, and in low- and high-expression sarcomas. Data are expressed as mean and standard error of the mean for each group.

All cases were examined for both cytoplasmic and membrane immunoreactivity. Cytoplasmic staining was evaluated on a semiquantitative scale, according to Kilpatrick et al with minor modifications,[20] and reported as 0 (no staining or staining in <10% of cells), 1+ (weak staining in >10% of cells), 2+ (moderate staining in >10% of cells), or 3+ (strong staining in >10% of cells). The presence of a membranous pattern of staining was recorded separately and scored as absent (no staining or weak staining in <10% of cells) or present (complete and/or incomplete staining in >10% of cells). Tumors with a cytoplasmic score of 3+ were considered to have high HER-2/neu protein expression.

Statistical Analysis

Statistical differences were calculated by Fisher's exact test. The *t*-test method was used to evaluate the differences between groups. Differences were considered statistically significant when P was <0.05.

RESULTS

HER2/neu mRNA Evaluation

All of the tissues analyzed contained detectable levels of HER2-neu mRNA. Six normal tissue samples (skeletal muscle) were used to establish basal level of HER2/neu mRNA. All the normal samples expressed very low levels of HER2/neu mRNA, ranging from 0.9 to 1.9 n (mean, 1.4 n). Among the 13 tumor samples, HER2/neu levels varied greatly, ranging from 2.1 to 24 n. Setting a cutoff level at 7.9 n (a value that represents the mean value of expression distribution of the SSs), 9 cases (69%) had low HER2/neu expression and 4 cases (31%) had high HER2/neu expression (Fig 1; Table 1). The difference between the 2 groups (low and high HER2/neu tumors) was statistically significant (P = 0.0004).

FIGURE 2. Immunohistochemical localization of HER2/neu in SS. (A) Positive control (breast cancer) showing typical strong membrane pattern of positivity. (B) Case 2, a biphasic SS, displaying very focal membrane staining limited to a gland. Inset: focal weak cytoplasmic positivity in the epithelial component of the same case. (C) Case 10, the epithelial component of this biphasic SS, displaying strong membrane pattern of staining. (D) Case 4, an epithelioid area in a monophasic fibrous SS, showing strong cytoplasmic positivity.

HER2/neu Protein Expression

Staining with HER2/neu antibody revealed a variable cytoplasmic and membrane staining pattern. Three tumors (23%) showed strong staining involving both the cell membrane and the cytoplasm (cases 4, 5, and 10); weak to moderate, exclusively cytoplasmic staining was observed in 7 cases (cases 1, 3, 6, 7, 8, 9, and 11). No staining was detected in 2 tumors (cases 12 and 13). In 1 case (case 2), a cluster of glandular structures representing <5% of the tumor showed weak cytoplasmic and very focal membrane staining, the latter limited to a single gland. The epithelial/epithelioid components exhibited stronger cytoplasmic staining compared with the spindle-cell component of the tumors. Membrane staining was predominantly incomplete and limited to the epithelial/epithelioid areas. All 3 cases with high immunohistochemical expression of HER2/neu (cases 4, 5, and 10) were grade III sarcomas, including 1 MF, 1 BF, and 1 MF-PD SSs. Examples of HER2/neu cytoplasmic and membrane staining are depicted in Figure 2.

Correlation of Molecular and Immunohistochemical Results

A strong, statistically significant association was present between protein expression, for both membrane and cytoplasmic staining, and HER2/neu mRNA levels ($P = 0.01$), although 1 case (case 6) displayed discordant results. Interestingly, this neoplasm showed high HER2/neu mRNA levels, whereas only weak staining, limited to the cytoplasm of a minority of tumor cells, was detected by immunohistochemical analysis.

HER2/neu Expression and Clinicopathologic Parameters

Both HER2/neu protein expression and mRNA levels were evaluated to establish the relationships to

TABLE 2. Correlation Between Clinicopathologic Features and HER2/neu Expression as Detected by IHC and RT-PCR

	HER2/neu					
	IHC			PCR		
Variable	L	H	*P* value	L	H	*P* value
Age (years)						
<40	3	3		3	3	
>40	7	0	NS	6	1	NS
Sex						
Female	8	0		7	1	
Male	2	3	0.03	2	3	NS
Tumor size (cm)						
<5	3	2		3	2	
>5	7	1	NS	6	2	NS
Histological grade						
II	3	0		3	0	
III	7	3	NS	6	4	NS
Histological type						
MF	5	1		5	1	
BF	2	1		2	1	
PD	3	1	NS	2	2	NS
Chemo/Radiotherapy*						
Yes	4	2		3	3	
No	5	1	NS	5	1	NS
Recurrence†						
Yes	5	0		5	0	
No	2	3	NS	1	4	0.02
Metastasis†						
Yes	3	0		3	0	
No	4	3	NS	3	4	NS

Abbreviations: L, low expression; H, high expression; NS, not significant; MF, monophasic fibrous; BF, biphasic; PD, poorly differentiated (including MF and BF with poorly differentiated areas).
*Information not available for case 7.
†Cases 7, 12, and 13 were excluded from the analysis.

clinicopathologic features, including local recurrence and metastatic disease. Two cases (cases 12 and 13) with follow-up less than 12 months and 1 case (case 7) for which clinical information was not available were excluded from the analysis of recurrences and metastatic behavior.

No correlation was observed between HER2/neu mRNA expression and age, sex, tumor size, tumor grade, histotype, and metastasis. A correlation between sex of the patients and HER2/neu protein expression was found. In fact, none of the female patients showed high HER2/neu protein expression ($P = 0.03$). Patients with high Her2/neu mRNA levels had a lower risk of recurrence than those with low Her2/neu mRNA levels ($P = 0.02$). None of the cases with high HER2/neu mRNA levels developed metastatic foci, although the small number of observations precluded reaching statistical significance ($P = 0.1$). Results are detailed in Table 2.

DISCUSSION

The present work provides the first combined molecular by real-time RT-PCR and immunohistochemical evidence that HER2/neu overexpression occurs in SSs. Our results indicate that this parameter may provide prognostic information and suggest that a specific therapy with humanized monoclonal antibodies against HER2/neu may be considered in a significant number of SSs.

The HER2/neu oncogene has been extensively investigated as a prognostic factor and more recently as a predictor of response to therapy. It has been demonstrated in breast cancer, where HER2/neu overexpression is usually associated with gene amplification,[21] and in other epithelial tumors, including ovarian, gastric, lung, and urinary bladder carcinomas.

HER-2/neu amplification/overexpression appears to be an early event in oncogenic transformation by interacting with other members of the HER family.[3] In breast cancer, it is involved in cell cycle and apoptotic pathways through the antiapoptotic effects mediated by p53 and p21 deregulation.[22,23]

Whether HER2/neu overexpression plays an important role in mesenchymal neoplasms remains controversial. An immunohistochemical study of sarcomas, using a monoclonal antibody, reported no evidence of immunoreactivity for HER-2/neu in 6 SSs as well as in other 197 mesenchymal tumors, with cytoplasmic reactivity observed only in 1 case of peripheral neuroepithelioma.[10] A recent investigation reported gene expression profiles of 41 soft tissue tumors with cDNA microarray analysis. Among these sarcomas, 6 monophasic SSs were characterized by a unique expression pattern of a cluster of 104 genes, including the epidermal growth factor receptor, which shows 50% homology with the HER2/neu gene.[24] These data also suggest that the erb-B receptor family plays a significant role in SS. It has been demonstrated that a variable number of osteosarcomas overexpress HER2/neu.[11-15] However, more recent studies[20,25,26] were unable to detect any HER2/neu gene amplification and/or overexpression using fluorescence in situ hybridization (FISH), RT-PCR, and immunohistochemistry.

Differences in the techniques used may play an important role and explain (at least in part) these discrepancies. HER2/neu alterations can be evaluated using different techniques including immunohistochemistry, FISH, Southern hybridization, Northern blot, and competitive, differential, or real-time PCR.[27] Immunohistochemistry is the most common method for detection of HER2/neu overexpression, but it is significantly affected by the sensitivity and specificity of the antibodies used, the type of tissue (frozen versus formalin-fixed), and the various interpretative criteria and scoring systems used to evaluate cases. Indeed, most studies of HER2/neu expression in osteosarcoma used immunohistochemical techniques, with different monoclonal or polyclonal antibodies. The discrepancy in results may stem from the use of different antibodies, as well as a lack of standardized evaluation.

For these reasons, to evaluate HER2/neu immunoreactivity in our study, we used a polyclonal antibody (Dako, Carpinteria, CA), arguably the most diffuse and thoroughly tested antibody for HER2/neu assessment. Furthermore, we investigated HER2/neu mRNA ex-

pression with real-time RT-PCR, because it has been demonstrated that mRNA levels correlates tightly with protein expression.[28] At present, real-time RT-PCR probably represents the most powerful tool for quantitative analysis, because it allows better internal control and reduction of sample contamination, and provides more objective results.[18]

We analyzed HER2/neu gene expression at the mRNA and protein level in 13 cases of SS. HER2/neu expression was found in all of the cases investigated, and mRNA content in the tumors varied from 2.1 to 24 n.

The variability of mRNA levels in SSs is reflected on heterogeneity of protein expression pattern as detected by immunohistochemistry. We found that HER2/neu immunoreactivity correlates strongly with mRNA levels. A convincing cytoplasmic immunoreactivity was documented with the polyclonal antibody in 10 of 13 sarcomas. Distinct membranous staining was observed in 3 cases, although it was never comparable to the positive breast control. It was predominantly incomplete and identified in the epithelial/epithelioid component of SS. Interestingly, all of the cases with strong cytoplasmic staining also exhibited a membrane-staining pattern.

In breast cancers, a membranous pattern of staining is thought to be specific for HER2/neu protein expression and correlated with gene amplification, whereas cytoplasmic staining is usually considered nonspecific.[29] However, cytoplasmic positivity for HER2/neu has been reported to be prognostically significant in other tumor types, including bladder, colon, pancreas, thyroid, and nasopharyngeal carcinomas, and even in breast cancer.[30-35]

Patients with high HER2/neu mRNA expression had a significantly lower risk of recurrence. Similarly, all of the cases with high HER2/neu expression did not metastasize, although this correlation did not reach statistical significance due to the small number of cases studied. These data suggest that HER2/neu plays a role in the biology of SS and that HER2/neu overexpression may be linked to a less-aggressive clinical behavior. Indeed, unlike many cancers where HER2/neu overexpression has been shown to correlate with poor prognosis, HER2/neu levels are linked to a more favorable clinical course in other malignant tumors, such as thyroid carcinoma and osteosarcoma.[15,34]

The molecular mechanisms responsible for the action of HER-2/neu in SSs are unknown. A possible interaction between HER-2/neu and the other members of HER family could be important in tumorigenesis. Derangements of other oncogenes, tumor suppressors, and apoptosis regulators have been described in SSs. For instance, many SSs have been shown to be diffusely positive for bcl-2 family proteins (bcl-2, bax, bcl-x, and bak). These members of the bcl-2 family are involved in the regulation of apoptosis in SS.[36] This raises the hypothesis that complex alterations in apoptosis-controlling mechanisms are present in these neoplasms, with HER-2/neu interacting with Bcl-2 family members. Further studies are needed to clarify the mechanisms of apoptosis in SS.

Depending on the size and location, the therapy of choice for SS is radical local excision or amputation. Whenever radical surgery cannot be performed, radiotherapy in concert with local excision is suggested in an attempt to avoid the need for amputation. Only recently has a study evaluated the possible role of chemotherapy in the treatment of SS.[37]

The role of molecular markers in predicting treatment responsiveness is currently the focus of extensive investigation. Breast cancer patients with high HER2/neu expression appeared to benefit from high-dose CAF (cyclophophamide, adriamycin, and 5-fluorouracil) therapy.[38] In our study, 3 of 4 patients with high HER2/neu expression received adjuvant chemotherapy with ifosfamide; these patients had a favorable clinical outcome. These data raise the possibility that HER2/neu may have value in predicting which patients are likely to respond to specific adjuvant chemotherapy regimens. Whether the favorable significance of HER2/neu expression depends on predicting clinical recurrence, response to chemotherapy, or both remains to be fully elucidated in SS patients.

To the best of our knowledge, this is the first report that shows expression of HER2/neu in primary SS by real-time RT-PCR. Elevated levels of HER2/neu mRNA and protein are found in a significant group of SS patients, and these levels appear to correlate with features of good prognosis. Furthermore, our results suggest that this mechanism of disease in SS may be the target of specific inhibitory therapies based on humanized monoclonal antibodies. Considering the small number of patients examined, further investigation is needed to confirm these preliminary findings.

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